

SFE-SFC-MS – Unified Chromatography

The Latest Addition to the Chromatography Toolbox

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The use of so-called 'supercritical' carbon dioxide for extraction and chromatographic separation is not a new approach, yet interest in this alternative technique is growing with the advance of state-of-the-art equipment and a steadily increasing amount of research and publications on the subject [1-4]. Highly complex samples originating in the clinical field, natural product research, food or environmental control facilities and academic institutes, challenge analytical researchers who are always striving to improve separation techniques and simplify sample pretreatment. The use of 'supercritical' CO₂ for both extraction and chromatographic separation can offer exactly that, more efficient extraction processes combined with shorter analytical run times, while simultaneously reducing solvent consumption. Furthermore, extraction selectivity can be carefully controlled by tuning the solvating power of the CO₂ through changes in pressure and temperature, making it faster, more efficient and more versatile than liquid extractions [5].

Supercritical CO₂ is a semi-fluidic state of carbon dioxide where it is held at or above its critical temperature (31.1°C) and critical pressure (73.8 bar). In the supercritical state fluid density is strongly affected by temperature and pressure and its physical and thermal properties are between those of the liquid and the gaseous phase [6]. Thus, under these conditions, the compressibility and the diffusion coefficient of the supercritical solvent are much higher, while viscosity is decreased compared to a pure liquid, and therefore it is far better suited to permeate penetrable solids. Supercritical fluid extraction (SFE) using supercritical CO₂ as extraction solvent is therefore faster and more efficient as well as being cheaper and more environmentally friendly than use of toxic organic solvents such as hexane, heptane or chloroform. In an online SFE-SFC approach the solid sample, a dried blood spot, food sample or polymer is homogenised and placed in an extraction vessel in the SFE unit (see Figure 1). The supercritical CO₂ is then introduced into the vessel, where static and dynamic extraction conditions can be adjusted in pressure and temperature, according to the sample requirements. As all samples are extracted under light-shielding, anaerobic conditions, decomposition even of labile compounds, that are easily oxidised, photo- or heat-labile can be avoided.

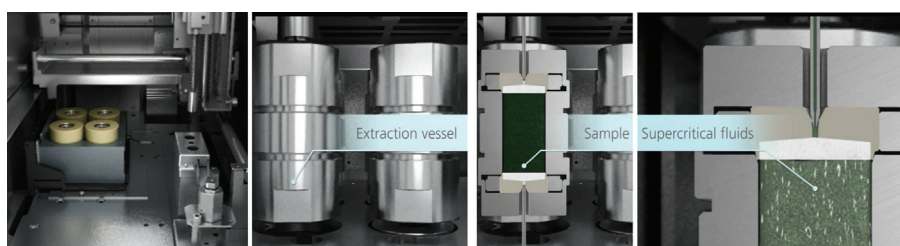


Figure 1: Supercritical fluid extraction in SFE unit

After extraction in the SFE unit the sample-containing CO₂ is introduced in the SFC flowline for chromatographic analysis by supercritical fluid chromatography (SFC) (see Figure 2).

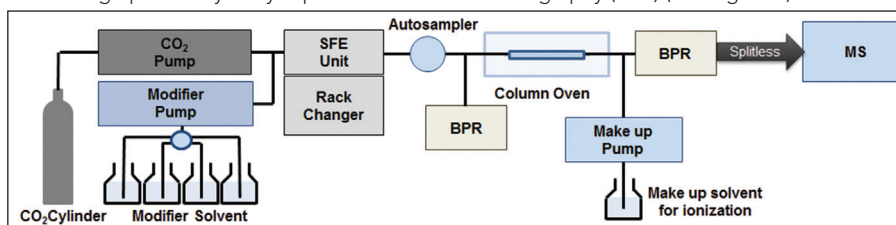


Figure 2: System configuration of Nexera UC: SFE-SFC-MS system

An application example is the extraction of the antioxidant stabiliser Irganox 1010 from a polymer sample. The solid, crushed polymer probe is enclosed in the extraction vessel and extracted with CO₂ at a pressure of 150 bar, kept constant with a back pressure regulator (BPR), and at a temperature of 50°C for seven minutes. Figure 3 shows the resulting SFC chromatogram after introducing the extract into the SFC flow-line.

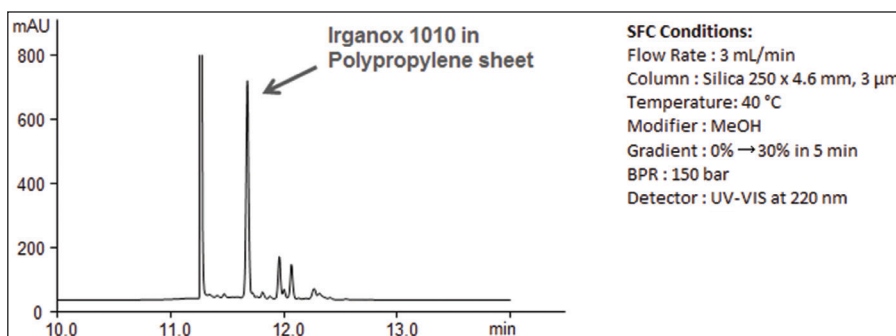


Figure 3: Online SFE and subsequent SFC analysis of polymer additive from polypropylene sheet

The fact that properties of supercritical CO₂ near the critical point change drastically with only small variations in temperature or pressure is advantageous in SFE, as extraction selectivity and efficiency can be easily adjusted. However, it also renders supercritical conditions a non-robust area to work in supercritical fluid chromatography, when changes in fluid density, hence selectivity, with column length and diameter, particle size and flow rate are unwanted [7].

CO₂ at its critical point is non-polar and elutropic solvent strength is often increased by using a polar co-solvent. As soon as such an organic modifier is introduced, the mobile phase is no longer truly supercritical, but the terminology is generally used regardless. These now “subcritical” conditions imply much lower changes in fluid density with small variation in pressure or temperature and are therefore a better, more robust area to work in for a reproducible separation (see Figure 4).

Many solvents are miscible with CO₂ (e.g. MeOH, EtOH (H-bonding), ACN (dipole)) and offer additional interactions. They, therefore, induce controlled changes in retention and provide a valuable tool in improving the selectivity of a separation.

Applications of SFE and SFC include recovery of organics from oil shale, separations of biological fluids, investigation of biomarkers,

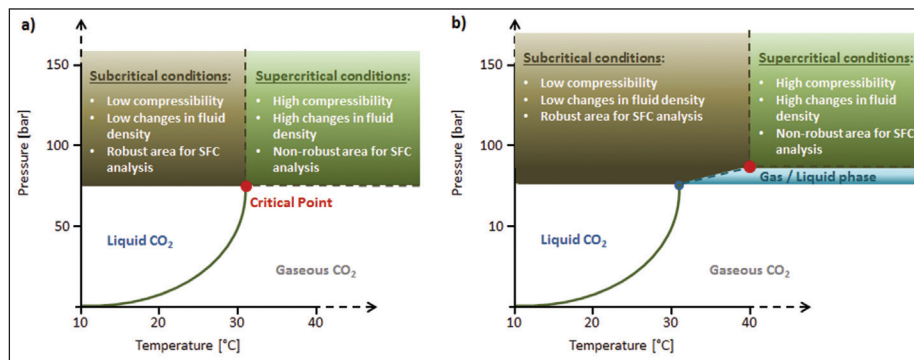


Figure 4: Phase diagram for a) pure CO₂ (critical point at 73.8 bar, 31.1°C) and b) 96.5 % CO₂ / 3.5 % MeOH (critical point at 83 bar, 40°C) [7]

selective extraction of fragrances, oils and impurities from agricultural and food products, pollution control, monitoring of pesticides in food products, additives in polymers, drug discovery in pharmaceuticals and biopharmaceuticals, environmental controls and cleaning validation [8]. This technology is also used in other areas and does provide the potential to become a standardised approach to sample extraction.

The use of supercritical CO₂ in industrial processes involving food or pharmaceuticals eliminates the possible presence of residual solvents which is always a concern when a ‘typical’ organic solvent is used. An additional benefit is the elimination of disposal costs associated with otherwise large volumes of organic eluents. SFE and SFC are therefore

faster, more efficient, cheaper and more environmentally friendly than other methods that require the use of large amounts of toxic organic solvents.

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